

AD _____

MIPR NO: 95MM5529

TITLE: Carcinogenesis of Depleted Uranium Fragments

PRINCIPAL INVESTIGATOR(S): Fletcher F. Hahn, D.V.M., Ph.D.

CONTRACTING ORGANIZATION: U.S. Department of Energy
Albuquerque, New Mexico 87185

REPORT DATE: February 1996

TYPE OF REPORT: Annual

19971014 088

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE February 1996	3. REPORT TYPE AND DATES COVERED Annual (26 Jan 95 - 25 Jan 96)	
4. TITLE AND SUBTITLE Carcinogenesis of Depleted Uranium Fragments			5. FUNDING NUMBERS 95MM5529	
6. AUTHOR(S) Fletcher F. Hahn, D.V.M., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Department of Energy Albuquerque, New Mexico 87185			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Several soldiers from the Gulf War were wounded by depleted uranium (DU)-containing shrapnel. There is concern that DU may be more hazardous than other shrapnel because of its radioactivity and known toxicity to the kidney. The risks associated with the long-term exposure to DU in this form are thought to be low, but are poorly understood. Predictions of risk are necessary to guide the medical management of soldiers with DU-bearing wounds both now and in the future. We are determining the carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive foreign-body fragments and assessing the potential for renal toxicity of DU fragments by correlating urine and kidney concentrations of U with time after implantation. DU fragments of differing sizes and shapes are being implanted in the subcutis of rodents to compare with results from animals implanted with inert metals. In this way a toxicity ratio can be determined that can be used to predict the expected response in humans from the known response of humans to relatively inert shrapnel. To date, a pilot study has been initiated to determine the important experimental design parameters for studying the foreign-body response using this test system in animals.				
14. SUBJECT TERMS Depleted Uranium (DU), Carcinogenesis, Rodents, Human Risks			15. NUMBER OF PAGES 31	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet official scanning requirements.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 80). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
EE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

NA Where copyrighted material is quoted, permission has been obtained to use such material.

NA Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

NA Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

YES In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

NA For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

 4/20/96
PI - Signature Date

TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION	1
A. Nature of the Problem	1
B. Background of Previous Work	2
C. Purpose of Present Work	6
II. BODY OF REPORT	7
A. Experimental Methods	7
B. Results	10
III. CONCLUSIONS	16
IV. REFERENCES	17
V. ADDENDA	22

I. INTRODUCTION

A. Nature of the Problem

Several soldiers in the U.S. Army have wounds containing depleted uranium (DU)-containing shrapnel. The risks associated with long-term exposure to DU-containing shrapnel in wounds are generally thought to be low, but are not known with precision. There is concern that DU, because of its radioactivity, may be more hazardous than other shrapnel.

Several soldiers who participated in Operation Desert Storm were wounded while in Bradley armored personnel carriers that were hit directly by armor-penetrating projectiles containing DU. Medical examination of these soldiers revealed elevated levels of uranium (U) in the urine at 1 year after exposure in two subjects, and shrapnel fragments visualized radiographically in at least one subject, documenting that measurable U is present *in vivo*. About 22 soldiers potentially bearing DU have been identified and are being followed to determine U excretion and possible biological effects (AFRRI, 1993). These data indicate that humans are being chronically exposed to U in both insoluble forms as DU fragments and soluble forms as dissolving U.

Quantitation of risk from exposure to U, particularly in the form of embedded fragments, is complex and involves both chemical and radiological components, as well as possible foreign-body effects. Because of the unique features of these exposures, it is not currently possible to reasonably predict the long-term risks to these soldiers from their U-bearing wounds. Such predictions are necessary, however, to guide the medical management of soldiers with U-bearing wounds both now and in the future.

To assess more confidently the carcinogenic risks associated with long-term exposure to DU-containing shrapnel in wounds, the following tasks are being addressed in this project:

- The carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive foreign-body fragments is being determined.
- Urine and kidney concentrations of U are being correlated with time after implantation of DU fragments.

B. Background of Previous Work

What do we know about the carcinogenicity of embedded fragments, either radioactive or nonradioactive?

Human data: There are very few situations involving people where cancer has resulted from radioactive fragments embedded in the tissues. Scar formation with central liquefactive necrosis has been reported in association with intradermal plutonium metal deposits in plutonium machinists (Lushbaugh *et al.*, 1967). The late effects of these deposits are not known, however, because excision of the deposited material was the treatment of choice in these accident cases.

Thorotrast, an X-ray contrast medium containing radioactive ^{232}Th as colloidal thorium dioxide, is known to cause tumors in the soft tissues of humans (Dahlgren, 1967). Inadvertent perivascular injection of this material results in the local formation of fibrous tissue (Thorotrast granulomas) in as many as 10% of the patients. In one study with incidence data, one metastasizing soft tissue sarcoma developed 30 years after injection in 142 patients with Thorotrast granulomas (Liebermann *et al.*, 1995).

The incidence of cancer associated with nonradioactive fragments or foreign bodies in the tissues is important information because of the lack of data on radioactive fragments. There are no epidemiologic studies and only a few case reports of foreign-body carcinogenesis in humans, however, which indicates that the overall incidence must be low. Recent case reports and a literature review (Jennings *et al.*, 1988; Lindeman *et al.*, 1990) showed only 40 cases of sarcoma associated with metallic foreign bodies, such as shrapnel, or metallic implants, such as prostheses. Another review notes that the risk of implanted medical devices must be very low because only about two foreign-body neoplasms are reported per year (Brand, 1994). A risk assessment of the cancers associated with implanted prostheses concluded that the risk must be small because the incidence of these cancers was low in the face of an increasing usage of such prostheses (Brand and Brand, 1980). The assessment also included failed attempts to isolate "precancerous" cells from tissues around the implants using cellular culture techniques, similar to those used in studies of foreign-body carcinogenesis in rodents.

Animal studies: In contrast to humans, foreign-body tumors have been frequently induced in rats (Nothdurft, 1956) and mice (Brand *et al.*, 1975). The development of sarcomas near the site of subcutaneous implantation of metal or polymer films in rodents is a well-described experimental result (Bischoff and Bryson, 1964; Brand *et al.*, 1975). Foreign-body carcinogenesis appears dependent on a specific sequence of four events: 1) cellular infiltration and proliferation during the acute reaction, 2) fibrosis of the tissue capsule surrounding the foreign body, 3) quiescence of the tissue reaction, and 4) clonal expansion of preneoplastic cells with direct contact on the foreign body.

The physical shape and characteristics of the implant, not the chemical reactivity, appear to be essential for tumor induction. Smooth-surfaced films, with a relatively large area, induce tumors with a high efficiency, while the same films minced into small fragments, but with the same surface area, have significantly reduced tumorigenicity (Brand *et al.*, 1975). The presence of the foreign body is essential only during the first months of the latent period. Recent work has shown that implantation of foreign bodies after injection of ethyl nitrosourea or after whole-body gamma irradiation also leads to increased sarcoma development (Moizhess and Vasiliev, 1989). Thus, foreign bodies with less than the critical surface area for carcinogenesis may act as promoters of subcutaneous carcinogenesis initiated by other agents, including radiation.

Thorotrast causes tumors in laboratory rodents, including tumors of the soft tissues (Bauer, 1948 as cited in Liebermann *et al.*, 1995). For example, 29 of 54 Chinese hamsters injected intravenously with a relatively high dose of Thorotrast (> 0.4 Bq/g) developed fibrosarcomas from perivascular leakage of some injections (Guilmette *et al.*, 1989). Plutonium fragments have been injected into the footpads of dogs to simulate the plutonium-contaminated wounds of plutonium machinists (Dagle *et al.*, 1984). The plutonium was translocated to the local lymph nodes where it caused fibrosis but no tumors.

The available literature provides little guidance for directly evaluating the carcinogenicity of DU fragments in soft tissues. Based on the cancer incidence data from people with nonradioactive foreign bodies, DU fragments do not appear to present a significant risk for causing cancer. However, there are indications from the foreign-body carcinogenesis studies in rodents and from the human experience with Thorotrast that radioactive foreign bodies may be more carcinogenic than nonradioactive foreign bodies.

What do we know about the renal toxicity of uranium?

Human data: Uranium protection standards for humans are based on the chemical nephrotoxicity of U (Voegtlin and Hodge, 1949, 1953; Tannenbaum, 1951). The basis for these standards was extensively reviewed in 1973 (Hodge, 1973; Spoor and Hursh, 1973). A critical level of U, at which renal damage could be expected, was defined as a peak concentration of 3 µg/g kidney, a judgement based on the best data available at the time. Subsequent experience indicates that adherence to the present limits for exposure to U, which are based on this critical level, appears to provide adequate protection against U nephrotoxicity (Spoor and Hursh, 1973). For example, a recent study of 31 workers exposed acutely to an accidental release of U hexafluoride resulted in renal U concentrations of 0.05 to 2.5 µg U/g kidney, but no workers had evidence of renal damage (Fisher *et al.*, 1990). However, another recent study of renal function in healthy U mill workers has shown a slight increase in urinary amino acids and proteins, indicative of reduced proximal renal tubular resorption (Thun *et al.*, 1985). These changes are consistent with nephrotoxicity and are found in workers with the highest potential for chronic exposure to soluble U. These findings raise concern for the possible renal toxicity of chronic low-level exposure to U.

Animal studies: The renal toxicity of U has been extensively studied in animals, particularly in rats (Haley, 1982; Haley *et al.*, 1982; Diamond *et al.*, 1987; Morrow *et al.*, 1982) and dogs (Morrow *et al.*, 1982; Eidson *et al.*, 1985). Dogs appear more susceptible to nephrotoxicity than humans and less susceptible than rats (Morrow *et al.*, 1982). A single injection of uranyl nitrate is a classic method for producing renal damage (Diamond, 1989). Necrosis of the terminal segments of the proximal renal tubule is characteristic for all species. At 1 month after a single exposure in rats, there is a thinning of

the proximal tubular epithelium, the result of regeneration of the necrotic epithelium (Haley, 1982). Studies of rats repeatedly injected with U have shown that renal lesions are first seen when the renal U burden is between 0.7 and 1.4 $\mu\text{g/g}$ and are most severe when the burdens are 3.4-5.6 $\mu\text{g/g}$. Repair is rapid; within 35 days the epithelium is normal (Diamond *et al.*, 1987). Studies of rats with constant 14-day perfusion of U (with osmotic pumps) have shown that renal toxicity is detected at renal U burdens of 1-2 $\mu\text{g/g}$ (Himmelstein, 1992). These results and others in humans (Thun *et al.*, 1985) have suggested that existing data on U nephrotoxicity should be reevaluated, particularly for chronic exposures (Leggett, 1989; Foulkes, 1990).

C. Purpose of Present Work

In this project, we are determining the risk of long-term DU-containing shrapnel in wounds. Two hypotheses have been formulated.

1. **Chronic low-level irradiation of tissues surrounding embedded fragments containing DU will increase the carcinogenic potency of the metal fragments.**

The objective of testing this hypothesis is to determine the hazard of radioactive fragments relative to nonradioactive fragments so that informed judgements can be made about the clinical management of veterans with DU fragments embedded in their soft tissues.

2. **The urinary concentrations of U are directly correlated with the renal concentrations of U and will reach a steady state after subcutaneous implantation of DU fragments.**

The objective of testing this hypothesis is to determine if the renal concentration of U reaches a steady state so that informed judgements can be made about clinical management of veterans who are chronically excreting U in their urine because of the slow dissolution of DU fragments embedded in their tissues.

Based on the information from these hypotheses, we will estimate the risks for DU-containing shrapnel in wounds.

II. BODY OF REPORT

A. Experimental Methods

1. **Relative Carcinogenicity of DU Fragments**

The specific aim of this study is to determine experimentally the relative carcinogenicity of radioactive DU and a nonradioactive inert metal. This relative carcinogenicity in rats will be used in a ratio so that the carcinogenicity of DU in humans can be estimated using the following relationship:

$$\{\text{DU toxicity/inert metal toxicity}\}_{\text{rat}} \sim \{\text{DU toxicity/inert metal toxicity}\}_{\text{human}}$$

This approach is similar to the toxicity ratio method previously described to compare the risk of radiation-induced bone cancer in dogs and humans (Mays *et al.*, 1986) and mice and humans (Finkel and Biskis, 1968). The information for inert metal toxicity in humans is foreign-body carcinogenesis data related to metals used for implants, stainless steel (SS), shrapnel, etc., (Brand and Brand, 1980; Brand, 1994; Galante *et al.*,

1991). Thorotrast is the positive control for radioactive material in rats. The responses in rats will be related to carcinogenic responses in humans exposed to Thorotrast deposited in subcutaneous locations (Liebermann *et al.*, 1995).

To experimentally determine the relative carcinogenicity, we are employing an experimental model of foreign body carcinogenesis that uses foils implanted in the subcutis of rodents. This model has been well characterized (Brand *et al.*, 1975), and the yield of subcutaneous sarcomas is related to the size, shape, chemical properties, and physical properties of the implant, and the genetic background, sex, and age of the host animal. We are taking advantage of the observations that films of plastics and metals larger than 5 mm square will cause subcutaneous neoplasms, but smaller films will not (O'Gara and Brown, 1967; Alexander and Horning, 1959). We will determine if the radioactivity of DU foils will increase the carcinogenicity of the larger foils or make smaller foils carcinogenic relative to the carcinogenicity of nonradioactive inert metal foils of similar sizes.

It is well known that rodents are much more sensitive to foreign-body carcinogenesis than humans (Furst, 1981). Thus, the direct test of carcinogenicity is rigorous and should not yield a false-negative result. On the other hand, a positive result cannot be extrapolated directly to the human situation, only the relative effect. To emphasize this point, a group of Thorotrast-implanted rats serve as a positive control group whose results can be compared directly to the sarcoma incidence of human patients with perivascular deposits of Thorotrast (Liebermann *et al.*, 1995). Rats with surgical manipulations similar to those used to insert the implants are sham controls.

The incidence of subcutaneous tumors will be compared among dose groups by using a Cox proportional hazards (CPH) model. These types of models take into

account not only the total incidence of tumors but also the times at which the tumors occur in order to obtain more power to test for statistically significant differences and to provide additional insight into the process of carcinogenesis. The relative risks estimated from the model for comparing DU to an inert metal in the rat are toxicity ratios of the two materials that will then be applied to humans using the observed foreign-body toxicity of inert metals such as SS. Because of the limited data available on foreign-body carcinogenesis in humans (e.g., Brand and Brand, 1980), we can only define an upper limit for toxicity. Data from rats injected with Thorotrast will also be analyzed with a CPH model to understand the role of radiation dose and provide another comparison with humans.

2. Renal Toxicity of Chronic Uranium Exposure

The specific aims of this study are to 1) determine the time course to achieve a steady-state renal DU concentration from an implanted DU source and 2) determine if toxicity is present at the steady-state concentration. In response to earlier reviews, this portion of the project is restricted to work that will obtain as much information as possible about the renal toxicity of DU in the animals that have implanted foils and are held for long periods. Accordingly, the scope of these studies is limited.

A pilot study of animals (implanted with DU foils) is being conducted to determine if the urinary excretion of U from implanted DU foils is sufficiently high enough above background to detect (Table 1). If elevated U concentrations can be detected in the urine, similar analyses will be conducted on a subset of animals in the carcinogenesis study. Urine and renal concentrations of U and renal toxicity tests would be conducted on the rats in the carcinogenesis study sacrificed at 1, 3, 6, and 12 months after implantation of DU foils.

Rats that are sacrificed are being examined for lesions, with particular attention to the urinary system. Kidneys are being examined histologically for lesions related to U toxicity. Tubular necrosis involving primarily the pars recta has been described in rats 5 days after injection of relatively high doses of uranyl nitrate (Haley, 1982). At 8 weeks after injection, focal necrosis of proximal tubular cells were noted (Haley *et al.*, 1982). Tubular regeneration began 1 week after injection, and by 2 months a majority of the tubules had regenerated. Thus, renal tubular lesions will be characterized in the DU rats for comparison with these previous reports. U content in the kidney and remainder of the carcass will be determined.

Laser phosphorimetry (Chemchek Model KPA-11) is being used to analyze tissue and urine samples for U. Prior to the U assay, all biological samples are dissolved by a combination of high temperature ashing (550°C) and acid digestion ($\text{HNO}_3 + \text{H}_2\text{O}_2$) using standard Inhalation Toxicology Research Institute (ITRI) methods. The method permits measurement of trace concentrations of U in complex solution mixtures. Experience at ITRI with the KPA-11 indicates a practical limit of detection for U(VI) of about 0.05 $\mu\text{g/L}$. This sensitivity should be adequate for measuring the U concentrations in both tissue and excreta samples for these studies.

B. Results

The first year of the project has been devoted to discussions and evaluations of the experimental approaches with consultants, rigorous evaluation of the experimental design, preparation of necessary protocols and obtaining the DU in the form necessary for the chosen approach. It has taken longer and cost more for the DU than originally anticipated.

A key feature of the experimental approach is based on the early work of Gerhard Brand, MD, and his colleagues (Brand *et al.*, 1975). Their work identified the important factors in inducing foreign-body carcinogenesis in rodents. Because of this work, Dr. Brand was contacted to consult on the project. Although semi-retired, Dr. Brand is still active and presented a seminar at the ITRI in March of 1995. His observations on the original experimental approach were invaluable.

In June 1995, Dr. Hahn visited AFRRI to discuss mutual interests in studies of DU toxicity. In preliminary studies in mice at AFRRI, intramuscularly implanted DU + .75% titanium (DUTi) pellets (1 mm in diameter \times 2 mm length) were shown to be more soluble over a 60 day period than anticipated. The surface of the DUTi pellets became "rough," and the tissue capsule that formed around the pellets adhered to the DU. In contrast, tantalum (Ta) pellets (used as an inert control metal) remained smooth, and the surrounding tissue capsule could be readily separated. If dissolution alters the physical characteristics of the DU foils to be used at ITRI, it will be necessary to reconsider the approach to determine the relative carcinogenicity of DU fragments compared with nonradioactive inert metal fragments. It has been demonstrated that the surface characteristics of implanted materials are important in foreign-body carcinogenesis in rodents (Brand *et al.*, 1975). Smooth surfaces appear to be essential for a foreign body to be carcinogenic in rodents. Therefore, if the surface of the DU foil to be used in the carcinogenesis study is altered in the rats and mice or if the foils dissolve in a matter of months, the long-term consequences may be changed from that expected based on the foreign-body carcinogenesis model in rats.

Based on these discussions and other new information, the following changes were made in approach:

- Pilot Study
 - BALB/c mice were added as a test animal (in addition to F344 rats)
 - DU + .75% Ti foils were added to compare solubility and durability with DU foils
 - An *in vitro* solubility test was included to compare DU and DU + .75% Ti
- Primary Study
 - Exposure groups were added to emphasize the concept of initiation and promotion in the experimental design
 - Minced foils were used, rather than foils of reduced size
 - Ta was used in place of SS as inert metal

A flow chart showing the revised studies and decision points is shown in

Table 1.

The original pilot study to investigate the urinary excretion of U from embedded DU foils was altered to include two species (F344 rats and BALB/c mice) to determine changes in surface characteristics of the foils and the histologic responses to implantation. BALB/c mice were added because this strain develops subcutaneous neoplasms in the shortest time after implantation (Brand *et al.*, 1977). In the original proposal only rats were included because of the interest in U effect on the kidney, work previously done primarily in rats. DUTi foils were added as test materials because this is the alloy actually used in penetrators and is the material being used in studies at AFRRI. The potential exists for differences in solubility between DU and DUTi.

The experimental design for the revised pilot study is shown in Table 2. The stability of the foils is being determined by the loss of foil weight during the study, the transfer factor from the implant site to the kidney, and the condition of the surface of the removed foil. The histologic reaction is being graded for relative amounts and character of inflammatory cell infiltrate, and amount and character of fibrosis.

The urinary excretion of U and concentration in the kidney are being determined, as noted previously in Experimental Methods. Results of this study will determine the stability of the DU and DUTi foils in the subcutis of both rats and mice and document the amount of urinary excretion of U. At the same time, *in vitro* studies on the solubility of DU, DUTi and U will determine and further document any differences in solubility.

Taken together, the results will be used to determine which foils and species should be used for the primary initiation/promotion carcinogenesis study. If there is breakdown of the foils or a continued inflammatory response to the foils in both species, a bioassay approach using intramuscular implantation will be used.

The experimental design for the primary experiment has been revised. As in the original proposal, it is based on the model of foreign-body carcinogenesis in the subcutis of rodents. The design has been modified to emphasize the idea of initiation and promotion coming from work on foreign-body carcinogenesis (Brand *et al.*, 1975; Moizhess and Vasiliev, 1989). In this model (Table 3), an initiation event occurs within 1–4 months after foreign body implant. At this time preneoplastic cells (as determined by a series of transplant experiments and use of marker chromosomes) are present on the surface of the foreign body. If a fully carcinogenic foreign body is removed before 3.5 months, no neoplasm develops.

The initiation is followed by a promotion stage in which a distinct fibrous capsule is formed around the foreign body, and the preneoplastic cells proliferate. After about 4 months neoplastic cells appear as clonal cells which are transferrable to another host. However, these transferred cells will not continue to proliferate without a promoting stimulus such as a foreign body. The presence of the foreign body, is important in perpetuating the foreign-body reaction leading to fibrosis. After about 5 months the proliferating cells become autonomous, creating a neoplasm. However, if inflammation is prolonged by mechanical irritation or sepsis, the tumor appearance is markedly delayed.

Based on this paradigm, solid materials can be made to be strong initiators/promoters (e.g., induce a high incidence of subcutaneous neoplasms) merely by having the proper size and shape (e.g., large, smooth surface area). They can also be made to be weak initiators or merely promoters by reducing the size and shape as has been done with relatively inert metals such as silver, plutonium, and gold (Nothdurft and Mohr, 1958). The key question is whether DU is a weak initiator in this model system like other inert metals or if it is a strong initiator, making it more hazardous than an inert metal. In rodents we predict, based on previous work, that DU or Ta in a foil as large as 15×22 mm will be both an initiator and promotor (Table 4). In a subcritical size (in a minced form), Ta should be only a promotor. We do not know the capabilities of DU in a minced form but if it does cause neoplasms by itself, then it is an initiator and promotor. If it causes neoplasms with minced Ta or more neoplasms when combined with Ta foils, it is a strong initiator. In this way, we will determine if DU is an initiator of carcinogenesis.

Thirty animals per group will be used and observed for 2 years; 20 animals added to each group will be sacrificed at 1, 3, 6, and 12 months after implantation (the

species to be determined from the pilot study). Sacrifice times were selected based on the study of Brand *et al.* (1975) on the stages of foreign-body tumorigenesis (Table 3). At 1 month after implantation, there should be an early foreign-body reaction with cellular infiltration and proliferation. At 3 months there should be a reactive fibrotic capsule around the foreign body and phagocytic inactivity. At 6 months there should be a quiescent foreign-body reaction and phagocytic inactivity. At 12 months the first neoplasms, originating in the fibrous capsule, should appear. These sacrificed animals will be used to document the time course of lesions and the intensity of the foreign-body reaction. U concentrations at the implant site and in the kidney and bone will be determined. Tissue samples from the implant sites and neoplasms will also be frozen for potential future molecular biology studies.

The animals on 2-year study will be observed at least twice daily and moribund or terminally ill animals euthanized. Once a week, surgical sites will be palpated for evidence of inflammation or onset of tumors. All surviving animals will be sacrificed once 90% of any one group has died or at 24 months, whichever occurs first. A complete necropsy will be performed with examination of all organ systems, paying special attention to the implant sites and the urinary system. Histological examination will be routinely performed on the implant sites, including site neoplasms, gross lesions that are potential metastases, and the kidneys. Neoplasms at the implant sites will be characterized with light microscopy and immunohistochemistry. Ultrastructural studies of the tumor cells have implicated a pluripotential mesenchymal cell type possessing morphologic characteristics consistent with cell types of the microvasculature as the preneoplastic parent cell (Johnson *et al.*, 1973). Thus, cell identifications will focus on endothelial cells, smooth muscle cells, and pericytes.

Concurrently with the initiation-promotion experimental protocol, a second, more conventional, carcinogenesis bioassay study using intramuscular injection of wires will be conducted (Furst, 1981). The experimental design for this study is shown in Table 5. DU wires (10 mm \times 0.5 mm diameter) will be embedded in the thigh muscles of F344 rats. Two doses of 1 or 5 wires will be used. As positive controls, Thorotrast (a colloid thorium dioxide solution) will be injected intramuscularly. As negative controls, SS wires will be injected. The specific activity of the solution is 80 pCi/g and has been used in previous studies at ITRI (Guilmette *et al.*, 1989). These animals will be held for 2 years in a manner similar to the previously described study.

This protocol most closely mimics what is seen in the exposure of the Gulf War veterans to DU-containing shrapnel. This is a simple, straightforward approach; however, little work has been reported that can be used for a basis of comparison using such a route of exposure.

III. CONCLUSIONS

The initial year of the project has been devoted to discussion with consultants on the experimental approach, rigorous evaluation of the original experimental design, preparation of necessary protocols and approvals, and locating a source and obtaining DU foils.

These evaluations have focussed on the experimented design and laid the ground work for an active second year of the project. The necessary foils for the pilot study are to be delivered in February 1996. It is anticipated that the pilot study will be completed and the primary initiation/promotion study and the carcinogenesis bioassay study will be initiated in the second year.

IV. REFERENCES

- AFRRI (Armed Forces Radiobiology Research Institute): *Protocol for Monitoring Gulf War Veterans with Imbedded Depleted Uranium Fragments*, Bethesda, MD, 1993.
- Alexander, P. and E. S. Horning: Observations on the Oppenheimer method of inducing tumors by subcutaneous implantation of plastic films. In *Ciba Foundation Symposium on Carcinogenesis: Mechanisms of Action* (G. E. W. Wolstenholme and N. O'Connor, eds.), Little, Brown & Co., Boston, pp. 12-25, 1959.
- Bischoff, F. and G. Bryson: Carcinogenesis through solid state surfaces. *Tumor Res.* 5: 85-133, 1964.
- Brand, K. G., L. C. Buoen, K. H. Johnson and I. Brand: Etiological factors, stages, and the role of the foreign body in foreign body tumorigenesis: A review. *Cancer Res.* 35: 279-286, 1975.
- Brand, I., L. C. Buoen and K. G. Brand: Foreign-body tumor in mice. Strain and sex differences in latency and incidence. *J. Natl. Cancer Inst.* 58: 1443-1447, 1977.
- Brand, K. G. and I. Brand: Risk assessment of carcinogenesis at implantation sites. *Plant Reconstr. Surg.* 66: 591-594, 1980.
- Brand, K. G.: Do implemented medical devices cause cancer? *J. Biomater. Appl.* 8: 325-343, 1994.
- Dagle, G. E., R. W. Bristline, J. L. Lebel and R. L. Watters: Plutonium-induced wounds in beagles. *Health Phys.* 47: 73-84, 1984.
- Dahlgren, S.: Effects of locally deposited colloidal thorium dioxide. *Ann. N. Y. Acad. Sci.* 145: 786-790, 1967.

Diamond, G. L.: Biological consequences of exposure to soluble forms of natural uranium.

Radiat. Prot. Dosim. 26(14): 23-33, 1989.

Diamond, G. L., R. M. Gelein, P. W. Morrow, B. J. Panner and R. B. Baggs (eds.):

Nephrotoxicity of Uranyl Fluoride and Reversibility of Renal Injury in the Rat.

NUREG/CR-4951, Prepared for U.S. Nuclear Regulatory Commission, Washington, DC, 1987.

Eidson, A. F., E. G. Damon, F. F. Hahn and J. A. Pickrell: Comparison of uranium retention in dogs exposed by inhalation to two forms of yellowcake. In *Occupational Radiation Safety in Mining*, Vol. 1 (H. Stocker, ed.), Canadian Nuclear Association, Toronto, Canada, pp. 261-264, 1985.

Finkel, M. P. and B. O. Biskis: Experimental induction of osteosarcomas. *Progr. Exp. Tumor Res.* 10: 72-111, 1968.

Fisher, D. R., M. J. Swint and R. L. Kathren (eds.): *Evaluation of Health Effects in Sequoyah Fuels Corporation Workers from Accidental Exposure to Uranium Hexafluoride.* NUREG/CR-5566, PNL-7328, Prepared for U.S. Nuclear Regulatory Commission, Washington, DC, 1990.

Foulkes, E. C.: The concept of critical levels of toxic heavy metals in target tissues.

Toxicology 20(5): 327-339, 1990.

Furst, A.: Bioassay of metals for carcinogenesis: Whole animals. *Environ. Health Perspect.* 40: 83-91, 1981.

Galante, J. O., J. Lemons, M. Spector, P. D. Wilson and T. M. Wright: The biologic effects of implanted materials. *J. Orthop. Res.* 9: 760-775, 1991.

- Guilmette, R. A., N. A. Gillett, A. F. Eidson, W. C. Griffith and A. L. Brooks: The influence of non-uniform α -irradiation of chinese hamster liver on chromosome damage and the induction of cancer. In *British Institute of Radiology Report 21 on Risks from Radium and Thorotrast* (D. M. Taylor, C. W. Mays, G. B. Gerber and R. G. Thomas, eds.), British Institute of Radiology, London, pp. 142-148, 1989.
- Haley, D. P.: Morphologic changes in uranyl nitrate-induced acute renal failure in saline- and water-drinking rats. *Lab. Invest.* 46(2): 196-208, 1982.
- Haley, D. P., R. E. Bulger and D. C. Dobyan: The long-term effects of uranyl nitrate on the structure and function of the rat kidney. *Virchows Arch. B. Cell Pathol.* 41: 181-192, 1982.
- Himmelstein, M. W.: *Physiologically-Based Toxicokinetic Modeling of Soluble Hexavalent Uranium in the Rat*. A dissertation submitted to the Division of Graduate Studies of the University of Cincinnati, 1992.
- Hodge, H. C.: A history of uranium poisoning (1824-1942). In *Uranium, Plutonium, Transplutonium Elements, Handbook of Experimental Pharmacology*, Vol. 36 (H. C. Hodge, J. N. Stannard and J. B. Hursh, eds.), Springer Verlag, New York, pp. 5-68, 1973.
- Jennings, T. A., L. Peterson, C. A. Axiotis, G. E. Friedlaender, R. A. Cooke and J. Rosai: Angiosarcoma associated with foreign body material. *Cancer* 62: 2436-2444, 1988.
- Johnson, K. H., H. K. Ghobrial, L. C. Buoen, I. Brand and K. G. Brand: Nonfibroblastic origin of foreign body sarcoma implicated by histological and electron microscopic studies. *Cancer Res.* 33: 3139-3154, 1973.

- Leggett, R. W.: The behavior and chemical toxicity of U in the kidney: A reassessment. *Health Phys.* 57(3): 365-383, 1989.
- Liebermann, D., H. Luehrs and G. Van Kaick: Late effects by paravascular thorotrast deposits. In *Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium* (G. vanKaick, A. Karaoglou, and A. M. Keller, eds.), World Scientific, Singapore, pp. 271-274, 1995.
- Lindeman, G., M. J. McKay, K. L. Taubman and A. M. Bilous: Malignant fibrous histiocytoma developing in bone 44 years after shrapnel trauma. *Cancer* 66: 2229-2232, 1990.
- Lushbaugh, C. C., R. J. Cloutier, G. Humason, J. Langham and S. Guzak: Histopathological study of intradermal plutonium metal deposits: Their conjectured fate. *Ann. N.Y. Acad. Sci.* 145: 791-797, 1967.
- Mays, C. W., G. N. Taylor and R. D. Lloyd: Toxicity ratios: Their use and abuse in predicting the risk from induced cancer. In *Life-Span Effects Studies in Animals: What Can They Tell Us?* (R. C. Thompson and J. A. Mahaffey, eds.), National Technical Information Service, Springfield VA, CONF-830951, pp. 299-310, 1986.
- Moizhess, T. G. and J. M. Vasiliev: Early and late stages of foreign-body carcinogenesis can be induced by implants of different shapes. *Int. J. Cancer* 44: 449-453, 1989.
- Morrow, P. E., L. J. Leach, F. A. Smith, R. M. Goloin, J. B. Scott, H. D. Belter, F. J. Amato, J. J. Picano, C. L. Yuile and T. G. Consier (eds.): *Metabolic Fate and Evaluation of Injury in Rats and Dogs Following Exposure to the Hydrolysis Products of Uranium Hexafluoride*, NUREG/CR-2268, RH, Prepared for Division of Health Siting and Waste Management, Washington, DC, 1982.

- Nothdurft, H.: Experimentelle sarkomauslösung durch eingeheilte fremdkörper. *Strahlenther.* 100: 192-210, 1956.
- Nothdurft, H. and H. J. Mohr: Sarkomerzeugung mit Fenterglas. *Naturwissenschaften* 45: 549, 1958.
- O'Gara, R. W. and J. M. Brown: Comparison of the carcinogenic actions of subcutaneous implants of iron and aluminum in rodents. *J. Natl. Cancer Inst.* 38: 947-957, 1967.
- Spoor, N. L. and J. B. Hursh: Protection criteria. In *Uranium, Plutonium, Transplutonium Elements, Handbook of Experimental Pharmacology*, Vol. 36 (H. C. Hodge, J. N. Stannard and J. B. Hursh, eds.), Springer Verlag, New York, pp. 241-270, 1973.
- Tannenbaum, A.: *Toxicology of Uranium*, McGraw-Hill, New York-Toronto-London, 1951.
- Thun, M. J., D. B. Baker, K. Steenland, A. B. Smith, W. Halperin and T. Berl: Renal toxicity in uranium mill workers. *Scand. J. Work Environ. Health* 11: 83-90, 1985.
- Voegtlin, C. and H. C. Hodge: *Pharmacology and Toxicology of Uranium Compounds*, Vols. 1 and 2, McGraw-Hill, New York, 1949.
- Voegtlin, C. and H. C. Hodge: *Pharmacology and Toxicology of Uranium Compounds*, Vols. 3 and 4, McGraw-Hill, New York, 1953.

V. ADDENDA

1. Acronym and Symbol Definition

CPH = Cox proportional hazard

DU = Depleted uranium

DUTi = Depleted uranium + .75% Titanium

ITRI = Inhalation Toxicology Research Institute

SS = Stainless steel

Ta = Tantalum

U = Uranium

Table 1

Flow of Studies and Decision Points

Pilot Study of DU or DUTi Foils in Subcutis of Rats or Mice			
Effects		Solubility	
<ul style="list-style-type: none"> • Foils break down • Foils initiate intense, protracted inflammation 		<ul style="list-style-type: none"> • Foils soluble • U detected in kidney 	
Yes	No	Yes	No
Initiate Carcinogenesis bioassay study	Initiate Initiation/promotion carcinogenesis study	Initiate U analysis in carcinogenesis study	Delete U analysis in carcinogenesis study

Table 2

Experimental Design for Pilot Study

Rodent	To be sacrificed at 30 days			To be sacrificed at 60 days			Total
	DU foils	DUTi foils	Ta	DU foils	DUTi foils	Ta	
Rats (F344)	5	5	4	5	5	4	28
Mice (BALB/c)	5	5	4	5	5	4	28
Total	10	10	8	10	10	8	56

A. Urine sampling for U analyses:

1. Number of animals: DU rats = 3; DU mice = 3; DUTi rats = 3;
DUTi mice = 3; Ta rats = 3; Ta mice = 3.
2. Sampling times: 1 week of acclimatization in metabolism cages.
DU/DUTi animals: 24 hour samples collected on days -2, -1, 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 60 (15 sampling times).
Ta animals: 24 hour sample collected on days -2, 7, 14, 28, 35 (5 sampling times).

B. Total urine samples: DU animals 90; DUTi animals 90; Ta animals 30 (n = 210).

C. Tissue samples (kidney and eviscerated-depleted carcass) for U analyses:

1. DU: rats 10; mice 10.
2. DUTi rats 10; mice 10.
3. Ta: rats 8; mice 8.
4. Total tissue samples = 56.

Table 3

Stages of Foreign-Body Carcinogenesis in the Subcutis of Rodents^a

Timing (months after implant)	Morphologic Observations	Cellular Neoplastic Progression	Carcinogenic Stage
0-2	Acute foreign-body reaction-inflammation	Preneoplastic "parent" cells present in capsule at 1-2 months.	Initiation
2-4	Subacute foreign-body reaction-fibrosis with distinct capsule	Preneoplastic clonal cells present in capsule initially then on foreign body	Initiation
4	Chronic foreign-body reaction-quiescent fibrosis	Preneoplastic clonal cells transferrable but foreign-body presence required	Promotion
5-24	Chronic foreign-body reaction-and sarcomata	Neoplastic clone cells-autonomous	Promotion

^aBased on Brand *et al.*, 1975 and Moizhess and Vasiliev, 1989.

Table 4

Experimental Design for Initiation/Promotion Study

Subcutaneous Implant ^a	# of Animals ^b		Phenomena ^c		
	Longevity ^d	Sacrifice ^e	Initiation	Promotion	Neoplasia
DU ^f (minced)	30	20	?	?	?
Ta (minced)	30	20	0	+	0
DU (minced + Ta	30	20	?	+	?
DU (foil)	30	20	+	+	++
Ta (foil)	30	20	+	+	++
Du (minced) + Ta (foil)	30	20	+	+	++??
Thorotrast ^g (sub Q)	30	20	+	+	++
Sham	30	10	0	0	0

^aFoils = metals - 5 x 22 x 0.2 mm; minced = foils cut into 10 pieces.

^bSpecies (F344 rat or BALB/c mouse) to be determined from pilot study.

^c+ = phenomenon present; 0 = phenomenon not present; ? = phenomenon presence unknown.

^dHeld for 2 year observation

^eHeld for sacrifice at 1, 3, 6 and 12 months.

^fDepleted uranium or DU + .75 Ti depending on outcome of pilot study.

^gThorotrast liquid (0.1 cm³) injected subcutaneously.

Table 5

Experimental Design for Carcinogenesis Study

Exposure ^a	Dose	# of Animals
DU wires (10 x 0.5 mm diam)	1 wire	30
DU wires (10 x 0.5 mm diam)	5 wires	30
Thorotrast	0.1 cm ³	30
SS wires	5 wires	30
None	None	30

^aIntramuscular implantation